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Growth performance, hematological parameters and lipid profile of mice treated with black seed oil and vitamin-E

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Abstract: *Nigella sativa* (Black seed) has been recognized as one of the most popular herbs in many parts of the world for centuries and used as folk medicine to cure different kinds of diseases. Vitamin E is well known for its antioxidative and anti-inflammatory properties, and has been studied in the prevention of cardiovascular disease (CVD). The present study was performed to determine the effect of black seed oil and Vit-E on growth performance and blood biochemistry in mice. A total of 40 mice weight between 25-27 gm were used for conducting the research. At first, the mice were randomly divided into 4 groups consisting 10 mice in each group. Group A served as the control group whereas Group B was administered black seed oil @ 0.5 ml/kg/day, Groups C was taken vitamin E @ 200 mg/kg/day and Group D was received both black seed oil @ 0.5 ml/kg/day + vitamin E @ 200 mg/kg/day respectively for 42 days. Result showed that, body weight of B, C and D group revealed a significant decrease than the control group ($p < 0.05$). Hemoglobin concentration (Hb) and Total Erythrocyte Count (TEC) showed a significant change in B, C and D group than the control group ($p < 0.05$). On the other hand, Total Leukocyte Count (TLC) and Packed Cell Volume (PCV) were insignificant. The results of biochemical test explored that serum cholesterol and triglyceride value decreased significantly whereas HDL increased significantly in black seed oil, Vit-E treated groups than the control group ($p < 0.05$). But the LDL showed no change after treatment. It could be concluded that the black seed oil and Vit-E can be used in the therapeutic strategy of obesity, anemia and coronary diseases.

Keywords: hemoglobin; cholesterol; HDL; black seed oil; vitamin E; mice

1. Introduction

The uses of herbal medicine have been increased greatly over the past three decades because this type of medicinal plants is locally available and cheap (Amin and Nagy, 2009). Nowadays use of medicinal herbs is the best solution to cure a disease as compared to other therapy and unhealthy products because of its natural properties, which are less toxic (Al-Attar and Wafa'a, 2010). Among these medicinal plants, black seed is one of them (*Nigella sativa*), that belongs to the family Ranunculaceae and is most extensively used worldwide for its therapeutic purposes (Aggarwal *et al.*, 2008; Kamal *et al.*, 2010). It is a native plant from the Mediterranean area and also found in other regions of the world such as in Saudi Arabia, Middle Eastern, North Africa, South Europe and Asia used for culinary and medical purposes by the Romans (Randhawa and Al-Ghamdi, 2002). The seeds of *Nigella sativa* showed richness and diversity in its chemical composition. Carbohydrates, proteins, amino acids, volatile and fixed oils are contained in the seeds. Thymoquinone proved to be the main active constituent of the volatile oil of the black seed (Gali-Muhtasib *et al.*, 2006). Pharmacological studies on *Nigella sativa* have found that it possessed analgesic properties, stimulate bronchodilation, hypolipidemic, anti-tumor,

diuretic, immunomodulation, hypotensive, anti-diabetic, histamine release inhibitor and antioxidative effects, also having anti-helminthic, anti-fungal (Rogozhin *et al.*, 2011), anti-bacterial (Halamova *et al.*, 2010), anti-cancer, and anti-inflammatory activities (Ayed and Talal, 2011). The thymoquinone has hypocholesterolaemia effects besides its potential in reducing the effect of triglycerides and LDL (Bamosa *et al.*, 2002). Anwar and Tayyab (2004) reported that *N. sativa* in the diet has a favorable effect on the lipid profile by lowering the TG, total cholesterol (TC) and low-density lipoprotein (LDL)-cholesterol and increasing the high density lipoprotein (HDL)-cholesterol in rats. The administration of 1ml/kg/day of *Nigella sativa* oil stimulated the sexual hormones secretion that led to improve protein synthesis, WBC count and decrease the serum cholesterol concentration in blood (Juma and Abdulrahman, 2011). On the other hand, Vitamin E is well recognized for its antioxidant activity, it is capable of stopping the propagation of potent oxidants formed during cellular metabolism or introduced as toxic chemicals. Disorders of blood lipid levels promote atherosclerosis which is recognized as a major risk factor for cardiovascular disease (CVD) such as stroke, coronary artery disease and peripheral vascular disease. (Mackey and Mensah, 2004). Vitamin E has eight different stereoisomers, of which the α -tocopherol is known to have the greatest biological activity. α -tocopherol is the most abundant lipid soluble antioxidant *in vivo* and acts as an important inhibitor of lipid peroxidation in membrane systems (Lin and Chang, 2006). It has been observed that α -tocopherol can scavenge peroxy radicals, singlet oxygen and superoxide anion radicals (Lii *et al.*, 1998). However, to date, little attention has been paid to the effects of black seed oil and Vit-E on blood biochemistry. Therefore, the present study was aimed to examine the effect of black seed oil and Vit-E on growth performance, hematological parameters and serum lipid profile in mice to frame an alternative therapeutic tool for cardiovascular disease (CVD).

2. Materials and Methods

2.1. Ethical statement

Animal care, preparation and experimental protocols were approved by the Animal welfare and Experimentation Ethics Committee of Bangladesh Agricultural University, Mymensingh-2202. [AWEEC/BAU/2020(33)].

2.2. Experimental animals

The mice used for this study were purchased from ICDDR'B, Dhaka. They were reared in a compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30) °C and relative humidity of 70-80% with natural day light.

2.3. Experimental design

In this study, total 40 Swiss Albino mice (*Mus musculus*), aged 24-26 days with an average body weight of 25-27 gm were used. At first, the mice were randomly divided into 4 groups consisting 10 mice in each group. Group A served as the control group and received the normal mice ration whereas Group B was administered black seed oil (0.5 ml/kg/day) for 42 days. The mice in Group C was taken vitamin E (200 mg/kg/day) and Group D was received both black seed oil (0.5 ml/kg/day) + vitamin E (200 mg/kg/day) respectively, for 42 days.

2.4. Management practices

The diet was prepared on daily basis and diet and water were supplied *ad libitum* in all groups. Initial body weight of each mouse was measured with the help of a digital balance. Body weight was taken on the first day and then we recorded body weight at 14 days' intervals until the end of the experiment. Mice cage were cleaned regularly and proper hygienic and sanitary measures were adopted during the experimental period.

2.5. Collection of blood and serum preparation

At the end of the experiment (42 days), blood samples were collected by sacrificing the mice. The mice were kept fasting overnight. Then the mice were placed one by one in an airtight container containing diethyl ether pre-soaked cotton. They were being looked over for insensibility and taken out from the airtight vessel and blood was collected directly from heart by a sterile syringe. About 1 to 1.5 ml blood was collected and transferred 0.5-0.75 ml blood into anticoagulant containing tube and the remaining half of blood was transferred to another tube without anticoagulant for serum preparation. As per conventional method serum was separated by centrifugation and stored at -20 °C until tested.

2.6. Hematological parameters

Hemoglobin (Hb), Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC) were performed as per standard method indicated by Ghai, 2012.

2.7. Serum biochemical studies

The biochemical parameters of serum like Lipid profile (Total cholesterol, Triglycerides, HDL cholesterol and LDL cholesterol) was performed at Professor Dr. Mohammad Hussain Central Laboratory in Bangladesh Agricultural University, Mymensingh-2200.

2.8. Statistical analysis

All data were placed and stored in Microsoft Excel- 2010 and imported to the software IBM SPSS Statistics 20 for analysis by one-way ANOVA followed by post-hoc Turkey's test. Because of using multiple comparisons, the corrected p value was calculated and adjusted at 0.01 and 0.05 considered for level of significance.

3. Results and Discussion

3.1. Effects of black seed oil and vitamin E on average body weight gain in mice

Average body weight gain in mice upon treated with black seed oil and Vit-E is shown in Table 1. After 42 days, the final body weight was 49.14 ± 2.12 gm, 52.71 ± 4.96 gm, 47.71 ± 2.06 gm and 45.71 ± 3.35 gm in group A, group B, group C and group D respectively. Black seed oil has lipid peroxidation and antiobesity activity due to the presence of phytochemical thymoquinone (Fararh *et al.*, 2002). Bano *et al.* (2009) reported that black seed oil decrease body weight by suppression of appetite that could be associated to the neural circuits which regulate catecholaminergic, serotonergic and peptidergic system or via circulating leptin hormone signaling the brains satiety center to produce hypophagic effects. It has been demonstrated that vit-E has strong neuroprotective and antiobesity effects in mice feeding a high fat diet (Fukui *et al.*, 2019).

3.2. Effects of black seed oil and vitamin E on hematological parameters in mice

Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. From the Table 2, we found that, Hb was 6.80 ± 0.20 gm%, 7.20 ± 0.20 gm%, 7.33 ± 0.12 gm% and 7.13 ± 0.31 gm% for group A, B, C and D respectively. Total erythrocyte count was 6.20 ± 0.50 million/mm³ for control group A but increased significantly ($p < 0.01$) 7.20 ± 0.28 million/mm³, 7.54 ± 0.14 million/mm³ and 6.52 ± 0.50 million/mm³ for group B, C & D respectively. On the other hand, ESR was 1.00 ± 0.00 mm in 1st hour in control group A and significantly decreased ($p < 0.01$) in group B (0.00 ± 0.00 mm in 1st hour) and group C (0.00 ± 0.00 mm in 1st hour) after that it increased significantly ($p < 0.01$) in group D (0.67 ± 0.58 mm in 1st hour). But there is no significant change for TLC and PCV values among these treated groups compare to control. Black seed oil may increase the erythrocyte count and haemoglobin concentration by an increased number of cells in bone marrow that reached advanced developmental stages and the accelerating effect on the cellular respiratory mechanism (Ebaid *et al.*, 2011). Mohamed and Awad (2008) reported that, black seed oil significantly elevated Hb, TEC and PCV and decreased MCH and MCHC. It has been proposed that vitamin E inhibits the oxidation of unsaturated fatty acids such as linoleic acid on the erythrocyte membrane, and the deficiency of this vitamin increases the hemolysis of red cells (Levander *et al.*, 1977). Present study showed vitamin E increased haematological parameters nearest to control level. This might be due to the supplemented vitamin E increase in oxygen carrying capacity of hemoglobin.

3.3. Effects of black oil seed and vitamin E on serum biochemical parameters in mice

Effect of black seed oil and Vit-E on lipid profile e.g. Cholesterol, Triglyceride, HDL-c and LDL-c in different groups of mice are presented in Table 3. For biochemical parameters, the cholesterol level was 247.25 ± 0.07 mg/dL in control group A, that was decreased significantly ($p < 0.01$) in group C and group D having the cholesterol level 205.00 ± 0.07 mg/dL and 229.00 ± 0.05 mg/dL while increased significantly ($p < 0.01$) in group B (264.50 ± 0.08 mg/dL). The triglyceride level was found 190.00 ± 0.04 mg/dL in control group A, whereas 148.25 ± 0.06 mg/dL, 103.50 ± 0.21 mg/dL and 119.00 ± 0.07 mg/dL in group B, C and D respectively with significant ($p < 0.01$) changes. The HDL was 148.50 ± 0.16 mg/dL for group A, while 170.00 ± 0.11 mg/dL, 120.25 ± 19.11 mg/dL and 150.75 ± 0.06 mg/dL for group B, C and D respectively with significant ($p < 0.05$) changes. The LDL concentration was 60.75 ± 0.08 in control group A, whereas 64.85 ± 0.23 mg/dL, 64.85 ± 0.23 mg/dL and 52.80 ± 0.25 mg/dL for group B, C and D respectively with no significant change. Consumption of black seed oil as a supplement decreased lipid profiles (TG, TC, LDL) while it increased the HDL-C levels,

compared to the placebo group (Ebaid *et al.*, 2011). The reduction in the lipid concentration by black seed oil may result from the hypolipidemic effects of oleic (Alman-Farinelli *et al.*, 2005) and linoleic acids (Wendel and Belury, 2006) major unsaturated fatty acids of the oil or from its effect on lipoprotein. Vitamin E is a natural component of the membrane lipid bilayer and thus helps to maintain membrane stability. The molecular and cellular effects of vitamin E have been explained either by acting as an antioxidant preventing damage to membranes or proteins and regulating their activity by specifically scavenging reactive oxygen species (Kalender *et al.*, 2004) or by interacting and regulating specific enzymes and influencing cellular structure such as membrane and lipid domains (Azzi, 2007). Vitamin E may effectively minimize lipid peroxidation in biological systems (Aldana *et al.*, 2001). Vitamin E is a major lipid soluble chain-breaking antioxidant having positive effects on lipid profile and strongly inhibits the propagation of lipid peroxidation. It can provide protection of lipoproteins against in vivo peroxidation by decreasing free radical oxidative damage to lipids. (Morel *et al.*, 2006; Sahin *et al.*, 2006). Fukui *et al.* (2019) and Zhao *et al.* (2016), they found that intake of vit-E has shown to significantly decrease the amount of serum cholesterol and to reduce obesity.

Table 1. Effects of black seed oil and Vit-E on body weight in mice.

Day	Day 1	Day 14	Day 28	Day 42
Groups	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control (A)	35.75 ^a ±1.67	41.50 ^a ±5.04	44.86 ^a ±2.48	49.14 ^{ab} ±2.12
Black seed oil (B)	36.63 ^a ±2.00	44.75 ^a ±2.87	46.57 ^a ±3.64	52.71 ^a ±4.96
Vit-E (C)	35.88 ^a ±3.76	41.38 ^a ±3.29	43.00 ^a ±3.37	47.71 ^b ±2.06
Black seed oil+Vit-E (D)	35.50 ^a ±2.39	35.75 ^b ±2.55	38.86 ^b ±3.29	45.71 ^b ±3.35
P value	0.838	0.001	0.001	0.005
Level of Sig.	NS	**	**	**

** Significant at 1% level (p<0.01); *Significant at 5% level (p<0.05); NS=not significant (p>0.05)

Table 2. Effects of black seed oil and Vit-E on Hb, TEC, TLC, ESR and PCV in mice.

Parameters	Hb (gm%)	TEC (million/mm ³)	TLC (thousand/mm ³)	ESR (mm in 1 st hour)	PCV (%)
Groups	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control (A)	6.80 ^b ±0.20	6.20 ^c ±0.50	7.74 ^a ±0.48	1.00 ^a ±0.00	24.00 ^a ±1.00
Black seed oil (B)	7.20 ^{ab} ±0.20	7.20 ^{ab} ±0.28	7.75 ^a ±0.40	0.00 ^b ±0.00	25.33 ^a ±1.15
Vit-E (C)	7.33 ^a ±0.12	7.54 ^a ±0.14	7.39 ^a ±0.57	0.00 ^b ±0.00	25.67 ^a ±0.58
Black seed oil+Vit-E (D)	7.13 ^{ab} ±0.31	6.52 ^{bc} ±0.50	7.72 ^a ±0.37	0.67 ^a ±0.58	24.33 ^a ±0.58
P value	0.078	0.01	0.737	0.006	0.132
Level of Sig.	*	**	NS	**	NS

** Significant at 1% level (p<0.01); *Significant at 5% level (p<0.05); NS=not significant (p>0.05)

Table 3. Effects of black seed oil and Vit-E on Cholesterol, Triglyceride, HDL and LDL in mice.

Parameters	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Groups	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Control (A)	247.25 ^{ab} ±0.07	190.00 ^a ±0.04	148.50 ^{ab} ±0.16	60.75 ^a ±0.08
Black seed oil (B)	264.50 ^a ±0.08	148.25 ^{ab} ±0.06	170.00 ^a ±0.11	64.85 ^a ±0.23
Vit-E (C)	205.00 ^b ±0.07	103.50 ^b ±0.21	120.25 ^b ±19.11	64.05 ^a ±0.20
Black seed oil+Vit-E (D)	229.00 ^{ab} ±0.05	119.00 ^b ±0.07	150.75 ^{ab} ±0.06	52.80 ^a ±0.25
P value	0.001	0.011	0.047	0.613
Level of Sig.	**	**	*	NS

** Significant at 1% level (p<0.01); *Significant at 5% level (p<0.05); NS=not significant (p>0.05)

4. Conclusions

In conclusion, the authors suggest that dietary supplementation with black seed oil and Vit-E favorably decreased body weight, cholesterol and triglyceride and also improved Hb, TEC and HDL level. Therefore, black seed oil and Vit-E may be regarded as a useful therapy for hyperlipidemia and obesity to fight against

Coronary heart disease (CHD). However, further studies are required to know its effects on hyperlipidemic patients and compare it's with hypolipidemic agents.

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Conflict of interest

None to declare.

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